ECOLOGY AND THERMAL INACTIVATION OF MICROBES IN AND ON INTERPLANETARY SPACE VEHICLE COMPONENTS

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INTRODUCTION

For the past several years dry-heat treatment has been specified by the National Aeronautics and Space Administration (NASA) as the preferred means for the terminal sterilization of spacecraft and for decontamination of spacecraft components. Bacillus subtilis var. niger spores have been used by most laboratories involved in this work as the model organism for developing such processes.

Recent studies show the presence of organisms highly resistant to dry heat in soil and fallout around assembly and industrial manufacturing areas (Kennedy Space Center experiments).

We have demonstrated the dry-heat survival characteristics of the Cape Kennedy isolate 4-6 (B. brevis) spores (produced on laboratory media) at temperatures of 112, 115, 118, 120, and 125 C with 1.2 µg of water per ml of headspace air (Thirty-eighth Quarterly Report of Progress). We have also shown the presence of hardy organisms from soil samples obtained from four geographical areas of the United States (Thirty-ninth Quarterly Report of Progress). A resistant fraction appears to occur in low numbers (probably 10^3/g) in a soil sample.

During this quarter we continued our investigation of 4-6 (B. brevis) spores in our closed tin can system at < 0.019, 5.47, 14.6, and 40.1% RH. We also compared the heat resistance characteristics of 4-6 B. brevis and B. subtilis var. niger spores at

100% RH and 100 C, and we compared their morphological characteristics by scanning electron microscopy.

I. EXPERIMENTAL

A. Heat resistance characteristics of 4-6 (B. brevis) spores at < 0.019, 5.47, 14.6, 40.1, and 125 C in a closed tin can system.

A portion of spores stored in double-distilled water at 4 C was insonated for 24 min. The insonated spores were diluted and a repeating dispenser was used to deliver 0.01 into each stainless steel cup for a concentration of about 10^5 to 10^6 spores per cup. These cups were placed in a dessicator jar containing phosphorus pentoxide and predried overnight. The predried, inoculated cups were arranged on perforated circular shelves and placed in tin cans 3 inches in diameter by 2.375 inches high (or 206 x 300). Each shelf contained 30 cups, and each can contained four shelves, for a total of 120 cups per can. The cans, lids, and contents were dried in a vacuum oven for 90 min at 45 to 50 C (at 1.5-inch Hg pressure absolute). To increase the drying rate, the oven was purged with dry nitrogen every 10 min for the first 70 min, followed by five consecutive purges of nitrogen with a vacuum cycle between each purge. After the cans, lids, and contents were dried, they were removed from the oven and cooled to about 30 C in the equilibration hood. Appropriate amounts of water were added in the center cups (B shelf) and then sealed immediately with an electric can sealer. The cans were then removed from

the equilibration hood, and the seams were soldered. Next the cans were heated at appropriate time intervals in an oil bath, and cooled in a refrigerated water bath. Spore survivors were assayed by the conventional plating method using TSA supplemented with 0.2% yeast extract and 0.1% soluble starch for 4-6 (B. brevis) spores, and TGE agar for B. subtilis var. niger spores.

B. Heat resistance characteristics of 4-6 (B. brevis) and B. subtilis var. niger spores at 100% RH and 100 C.

The procedure is similar to that described in section I.A., except for the treatment of B. subtilis var. niger (i.e., inoculated cups were not predried in the dessicator overnight).

C. Scanning electron micrographs of nonheat-treated 4-6 (B. brevis) and B. subtilis var. niger spores.

Two Bacillus species were examined in this study. From a spore stock suspension, approximately 1×10^9 spores per ml were mixed thoroughly in a 2% glutaraldehyde fixative and placed in a 5-ml screw-cap vial.

Scanning electron microscopy services have been provided by Dr. P. S. Lin of the Radiobiology Division, Tufts University School of Medicine, Boston, Massachusetts.

II. RESULTS AND DISCUSSION

A. Heat resistance characteristics of 4-6 (B. brevis) spores at < 0.019, 5.47, 14.6, 40.1, and 125 C in a closed tin can system.

The dry-heat survival curves of 4-6 (B. brevis) spores at different relative humidities (< 0.019, 5.47, 14.6, 40.1, and 125 C) are shown in Fig. 1. This organism is particularly resistant to thermal inactivation. Maximal heat resistance is about 5% RH, which is similar in response to B. subtilis var. niger spores. The resulting population appears to be heterogeneous with respect to thermal inactivation. All inactivation curves showed deviations from linearity. The magnitude of this nonlinearity is seen at 5.47% RH. The resultant fraction occurs in about 1 in 1,000, a rate that is similar to the one for organisms found in soils. Significant thermal inactivation occurs at 125 C under dry conditions and under high humidity (40.6% RH). The results also show that for all practical purposes, this organism is unaffected by the Viking sterilization cycle.

B. Heat resistance characteristics of 4-6 (B. brevis) B. subtilis var. niger spores at 100% RH and 100 C in a closed tin can system.

Figure 2 shows inactivation curves of 4-6 (B. brevis) and B. subtilis var. niger spores heated at 100 C and 100% RH. In both species, spores were very susceptible at 100% RH.

C. Scanning electron microscopy of 4-6 (B. brevis) and B. subtilis var. niger spores.

Figures 3a and 3b show the ellipsoidal shape of nonheat-treated 4-6 (B. brevis) and B. subtilis var. niger spores. Both figures show characteristic ridge-like structures running longi-

tudinally along the spore axis, and it appears that B. brevis spores are slightly larger than B. subtilis var. niger spores.

III. CONCLUSION

Our findings on the dry-heat resistance of 4-6 (B. brevis) are consistent with those found in our work with soils--a very heat-resistant fraction occurs in about 1 in 1,000 soils.

Because hardy organisms are naturally present in soil and fallout, better techniques for their inactivation are needed.

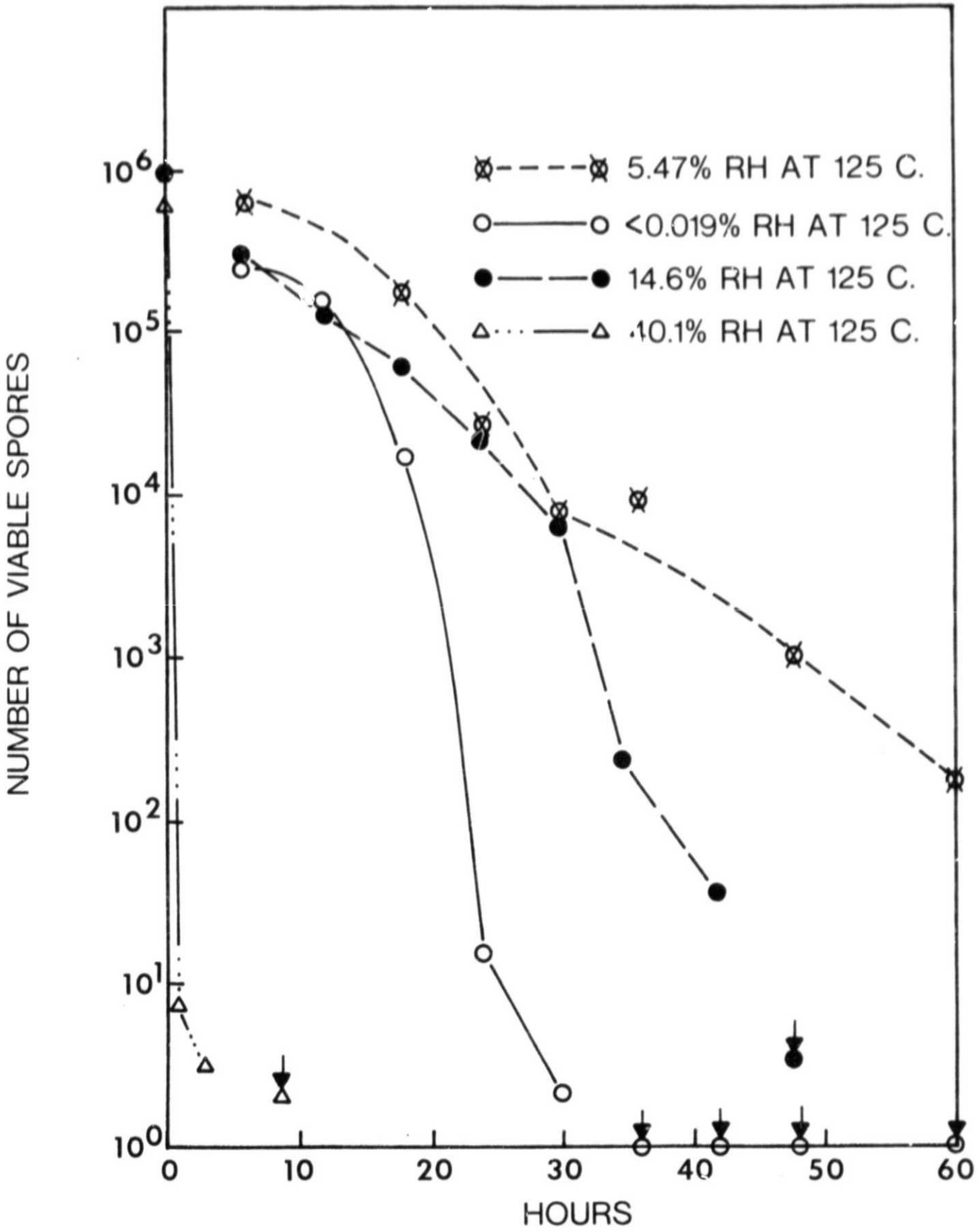

FIG. 1. INFLUENCE OF RH ON THE INACTIVATION OF 4-6 (B. BREVIS) AT 125 C.

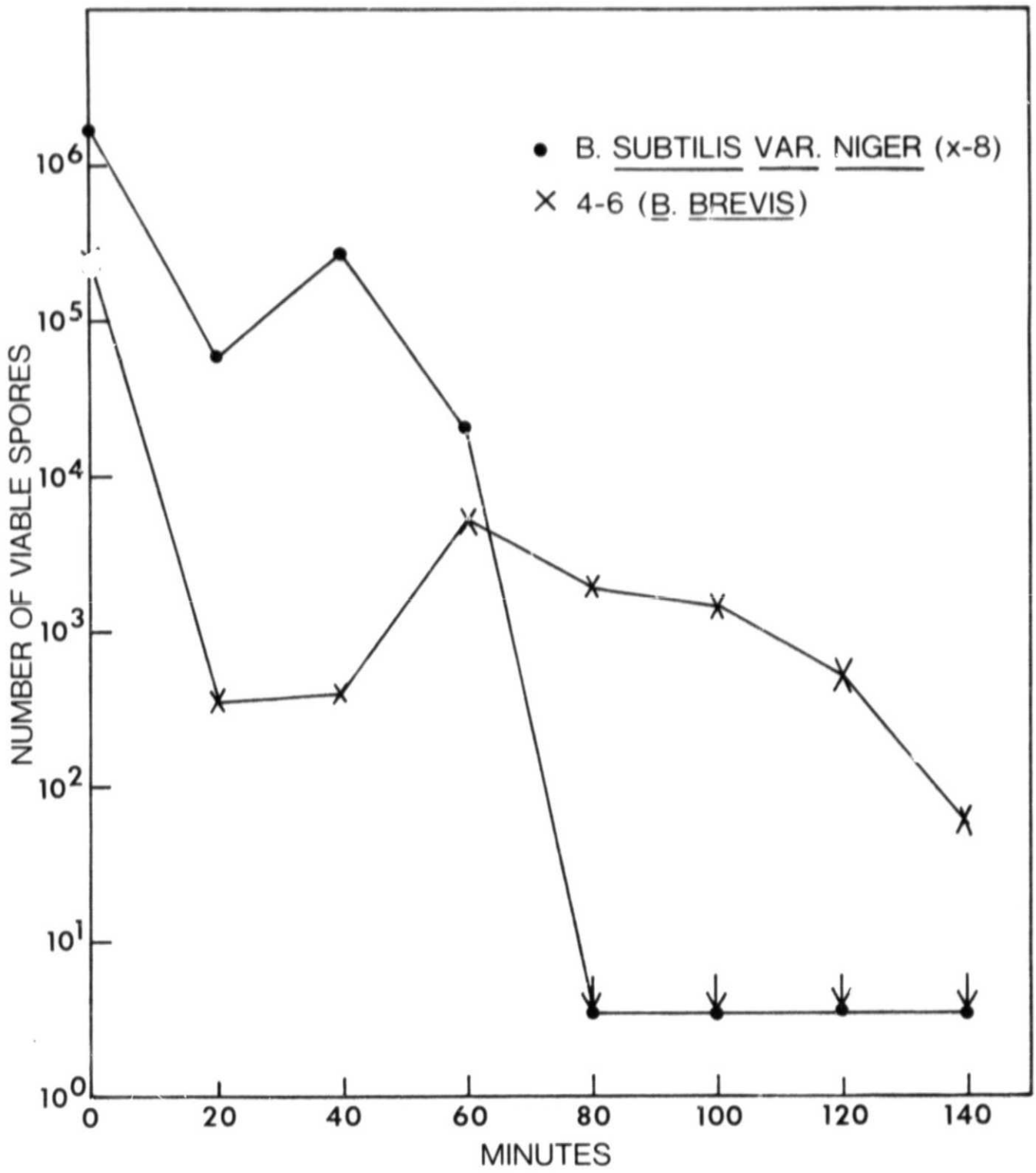

FIG. 2. THERMAL RESISTANCE OF B. SUBTILIS VAR. NIGER AND 4-6 (B. BREVIS) AT 100% RH AND 100 C.

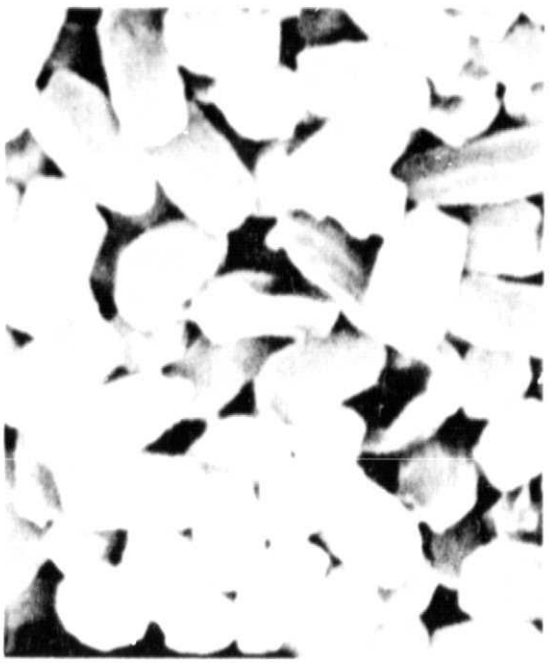

FIG. 3a. SCANNING ELECTRON MICROGRAPH OF B. BREVIS x 10,000.

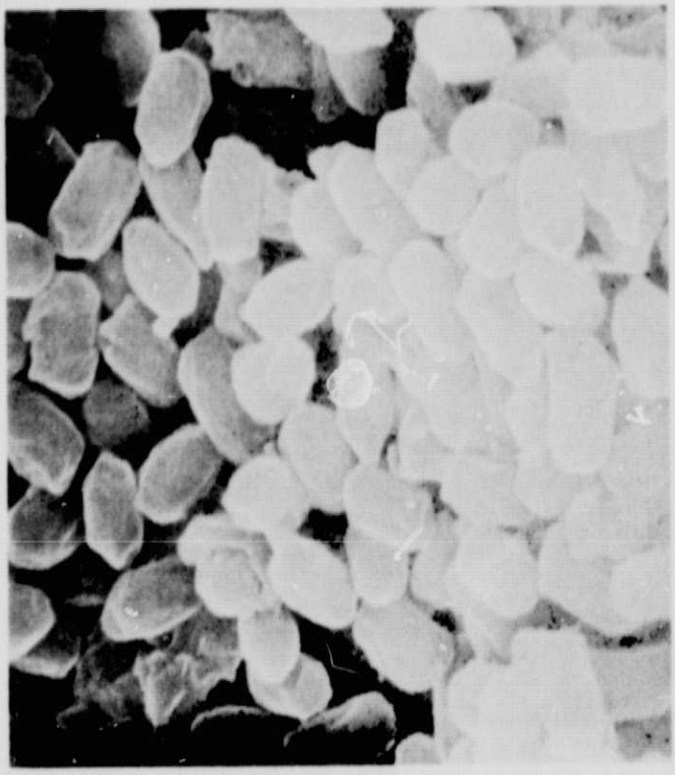

FIG. 3b. SCANNING ELECTRON MICROGRAPH OF B. SUBTILIS VAR. NIGER x 10,000.